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A study of the elimination of phosphorus following its ingestion in organic and inorganic forms

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A STUDY OF THE ELIMINATION OF PHOSPHORUS FOLLOWING ITS INGESTION IN ORGANIC AND INORGANIC FORMS

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INTRODUCTION

Because of the important role played by phosphorus in growth, metabolism, maintainance of blood neutrality, and the like, many investigators have attempted tofind that form of phosphorus best suited to supplement a phosphorus deficient diet.

There has been considerable discussion and disagreement as to the comparative nutritive values of phosphorus in its organic and inorganic forms. Some investigators have claimed that inorganic phosphorus may be used to the exclusion of organic phosphorus in supplying all the needs of the animal body. Other investigators have taken the middle ground with regard to the matter stating that organic phosphorus is superior, but in all probability either may be used. A third group have taken the stand that at least a small part of the requirement must be in the organic form.

With these facts in mind the present simple investigation was undertaken. An attempt was made to determine the relationship between ingested phosphorus, both organic and inorganic, and the urinary excretion of phosphorus. Three experimental periods were carried out in which organic phosphorus was ingested in the form of egg yolk and casein (cottage cheese). Inorganic phosphorus was similarly ingested as hypophosphites (Compound Syrup of Hypophosphites) and as disodium phosphate.

REVIEW OF LITERATURE +

General Discussion

About one percent of the body consists of phosphorus. Phosphates are widely distributed and are excreted even after long fasting. The excretion does not fall off rapidly, but follows parallel to the nitrogen, representing not only metabolism of preexisting salts, but metabolism of body tissues as well.

"Phosphorus is structurally a constituent of every cell nucleus and cellular structure, promenent in the skeleton, milk, sexual elements, glandular tissue, and nervous system. Functually it is involved in cell metabolism, multiplication, activation and control of enzyme actions, maintainance of the neutrality of the organism, conduct of nerve stimuli, relation to osmotic pressure, surface tension, and imbibition of water by colloids, movement of liquids, maintainance of proper liquid content of the tissues, cell movements, absorption and secretion."

The insoluble phosphates are found in bone, while the soluble are found in blood and protoplasm. They play an extremely important part in maintaining the neutrality of the blood through their buffer action.

Human bones contain about 600 grams, the muscles 56 grams, and the nerves 5 grams of phosphorus. Therefore the metabolism of bone tissue must exert considerable influence on the phosphorus output. The soluble phosphates of blood and tissue are constantly eliminated by the formation of acid phosphates in neutralizing acids.

+ The following review of literature consists, for the most part, of a secondary review of material collected from primary sources by Forbes and Keith (18). The quotations refer to the immediate source from which they were obtained and not to the primary reference.

The phosphorus compounds which occur in the body and in foods may be grouped into four classes.

1. Inorganic phosphates, amoung which potassium phosphate is the most abundant. It is found in food,fluids, and the soft tissues of the body. Calcium phosphate is the chief inorganic constituent of bones.

2. Phosphorus containing proteins, including the nucleo-proteins of cell nuclei, the lecitho-proteins, and the true phospho-proteins such as casein or caseinogen of milk and ovovitellin of egg yolk.

3. Phosphatids, phospholipins, or phosphorized fats - including lecithins, lecithans, kephalins, etc. - which occur in large quantity in brain and nerve tissue, and in other cells and tissues of animals and plants. These constitute about two thirds of the phosphorus in the egg yolk.

4. Phosphoric acid esters of carbohydrates and related substances, inositol and its natural salts. The calcium and magnesium salts of phytic acid (inositol phosphoric acid, $C_6H_{24}O_{24}P_6$) are found in many vegetables.

The phosphorus dealt with in foods is partly inorganic and partly organic in combination with proteins, carbohydrates, and fats. In digestion and metabolism the phosphoric acid radicals are split off, and ultimately, nearly all of it leaves the body as inorganic phosphates. The extent of cleavage in the digestive tract, and the amount of organic phosphorus which is absorbed is still a subject of investigation.

Marcuse (1) Steinitz, |2) Zadic, (3) and Leipziger (4) studied, by metabolism effects on dogs, the nutritive value of phosphoproteins when fed to the exclusion of phosphates, and when contrasted with equivalent amounts of phosphorus and nitrogen fed in the form of a mixture of inorganic phosphates and simple proteins. Casein and ovovitellin were the phospho-proteins used, and myosin and edestin were fed with inorganic phosphates. Sumarized by Rohmann (5) the results as a whole showed a striking difference in favor of the phospho-proteins as against the mixture of simple proteins with inorganic phosphates. The storage of nitrogen was also more promenent in the former case.

Ehrström (6) and Gumpert (7) working with men found that a smaller amount of phosphorus will maintain phosphorus equilibrium when taken in the form of casein than when taken in the form of dicalcium phosphate or as meal (potassium phosphate).

Keller (8) found that storage of phosphorus in young children was favored by food like milk, which contained a liberal supply of phosphates in addition to organic phosphorus: and Von Wendt (9) found that loss of phosphorus occuring with a diet poor in phosphorus could be reduced by the addition of dicalcium phosphate. The phosphorus of human milk occurs largely in organic forms, that of cows milk in inorganic forms.

In general the more recent investigations favor the view that the body can use inorganic phosphorus for all its requirements.

In 1909 Hart, McCullum, and Fuller (9) showed that, with young pigs on a diet too poor in phosphates, the deficit could be made good by feeding phosphates as well as by feeding organic phosphorus. In 1910 McCullum (9) reported that other things being equal the phosphorus requirements can all be satisfied by inorganic phosphorus.

According to Sherman (9) "there is no quantitative measure of differences in nutritive value as between different forms of phosphorus. If differences in nutritive value between the different groups of phosphorus compounds exist, they are doubtless in favor of the phosphoproteins and phosphatids and are more significant for the growing than for the full grown organism."

The amount of phosphorus excreted is influenced by the metabolism of the nucleoproteins,bone metabolism,and the amount of food taken in. The elimination of metabolized phosphorus is partly through the kidneys and partly through the intestinal wall. Foods rich in calcium, which tend to give an alkaline ash, increase the amount which is eliminated through the intestinal wall.

The phosphorus requirement can not be estimated from the urine as the results are too low. The phosphorus out put is influenced not only by the amount of phosphorus in the food, but also by the rate of metabolism during the preceeding days. In order to allow a sufficient margin of safty, the standard should be about 1.44 grams per 70 killograms of body weight per day. (9)

Bodansky (10) says that "while there is a possibility that a certain amount of phosphorus in organic combination is assimilated, most of the phosphorus is probably absorbed in the form of inorganic phosphates. At least it is certain that even in the growing animal the phosphorus may be supplied exclusively from inorganic sources."

"It is probably correct to assume that the phosphorus in bones is in equlibrium with its environment, and that any loss by solution is promptly made good."

"Muscular activity increases the excretion of phosphorus, this being associated with increased carbohydrate metabolism, and the liberation of phosphoric acid from combination as hexose phosphate." (10)

Phosphoric acid plays an important part in relation to the oxidation of sugars in the animal organism. Work done by Andove and Wagner (11), Viennese investigators, and confirmed by Kay and Robinson (11) showed that a hexose phosphate was formed as an apparently necessary preliminary step to the utilization of sugar by the tissues.

Harden and Robison (11) in working with the hexose phosphate of fermentation mixtures isolated a hexose monophosphate distinctly different from Neuberg's ester. It formed soluble calcium or barium salts, which were deposited as the phosphates by enzymatic action. This led to experiments which proved that the reaction was related to the deposition of calcium in bone formation, since the cartilage actually possesses the power to hydrolyse the monophosphoric ester.(11)

Grandis and Manini (12) by microchemical methods believed that they found evidence that the phosphorus of ossifying cartilage is converted into an inorganic form which then takes up calcium from the blood. Pacchioni (13), however, believed that calcium entered the cartilage already combined as a phosphate. (14)

Up to 1909 the belief was held that organic phosphorus compounds were assimilated directly and were therefore preferable to inorganic phosphates as the source of phosphorus. However, Fingerling and Gregerson (15) have both shown that organic phosphates can be synthesized in the animal body from inorganic phosphates. Feeding experiments do not show in what form the organic phosphorus is assimilated, that is, whether it is broken down previous to assimilation or not. At any rate phosphorus is excreted in the urine almost entirely as inorganic P_2O_5, whether the intake be organic or inorganic. Only a small quantity is excreted in the organic form, the composition of which is not known, although it is believed to be glycero-phosphoric acid.

The author (15) finds that except for phytic acid the organic phosphorus compounds are all hydrolysed by the intestinal mucosa. He concludes that the value of organic foodstuffs depends entirely upon the nature of the organic matter with which the phosphorus is combined.

Numerous metabolic studies have led to unsatisfactory results. Some investigators find the evidence from this kind of study to point to a more complete absorption of organic phosphorus than inorganic, while others find no difference in the results. The difficulty of drawing conclusions from such work and the conflicting results are not supprising when the many factors concerned are considered. The amount of calcium ingested directly influences the amount of phosphorus excreted. The more calcium, the greater the amount of phosphorus eliminated by the intestine at the expense of the kidneys. Too short a period of observation and the fact that the ingested phosphorus may be excreted very slowly have vitiated many experiments. If the organic phosphorus compounds possessany particular value over the inorganic, it is probably due to their containing traces of material necessary to normal nutrition, which are probably found in the normal diet. (16)

Forbes and Keith (18) found that after the ingestion of ordinary food, the inorganic phosphates are absorbed as such.

"The organic phosphates are absorbed in part without change, in part, after partial cleavage, and in part, after complete separation into their simpler groups with the liberation of phosphorus as inorganic phosphoric acid."

"Since the phosphorus of food is mostly in completely oxidized form there can be no significant change in the state of oxidation during metabolism. There is evidence of some capacity, however, for animals to oxidize the less completely oxidized compounds, as is observed by Heffter (19) who states that in healthy animals phosphorous acid is completely oxidized. He states also that pyrophosphates and hypophosphates are excreted unchanged, while metaphosphates are completely changed into the ortho form."

"The circumstances which determine whether phosphates shall be eliminated through the urine or the feces are first, the reaction of the alimentary tract as determined especially by the hydrochloric acid and the carbonates; second the nature and relative amounts of other mineral elements, especially the bases present in the digestive tract, as effecting the solubility of the phosphates; third, after the absorption of the phosphates from the alimentary tract, all of those circumstances which control the secretion of phosphates into the digestive tract, and their reabsorption from the same; fourth, the many circumstances which effect the reaction of the blood serum, and the amounts and proportions of the other salts present, as effecting the form and therefore the solubility of the phosphates, the more readily soluble tending to go out through the urine; and fifth, the species of animal involved."

It has been found that the principal secretion of the phosphorus was in the saliva and the secretion of the abomasum, and its largest absorption was from the small intestine.

"Phosphates are supposed to be excreted into the intestine as mono and di salts. Here the di salt decomposes into the mono and tri salts, and the latter,further, with the taking up of the elements of water, into the basic phosphate and acid. Since of these phosphates only the mono salts can be considered as relatively easily soluble in the weakly alkaline intestinal juice,their reabsorption takes place principally in this form, though to some extent also as the di salt."

Influence on Digestion

Finzi (20), 1903, determined that an intravenous injection of neutral sodium phosphate caused an increased secretion and an increased phosphate content of the saliva.

Ferrata and Moruzzi (21), 1907, demonstrated that a food containing phosphorus increased the lecithin content of the intestinal mucosa of the dog, while the nucleoproteids were increased following the taking of food of any kind.

Roger (22), 1908, found that the amylolytic power of the saliva decreased following the addition of uranium acetate,and completely disappeared with complete precipitation of the phosphorus. Addition of sodium phosphate caused the amylolytic power to reappear.

Loeb (23), 1910, showed with a dog that the phosphate content of the urine was reduced, temporarily, by the taking of food, apparently because of its utilization in the preparation of the digestive juices.

"Lisbonne (24),1911, reports that the phosphates do not activate salt-free amylases of the saliva and pancreatic secretion as do the chlorides."

" W. Löb (25),1911, demonstrated that phosphates stand in important relationship to the utilization of sugar. Glycolosis, which is favored by hydroxyl ions is accelerated by the presence of the phosphate ion; with constant hydroxyl ion concentration the acceleration rises with the absolute amount of phosphate present. Lecithin or glycerophosphates hinder phosphate glycolosis."

"The above notes show that phosphates stand in such a significant relation to digestion and the utilization of food that their excretion into the alimentary tract must be regarded as of vital consequence."

Methods of Urinary Elimination

"Maly (26),1876, shows that acid constituents diffuse and dialyse more rapidly than alkali from such mixtures as the blood, this depending on the greater solubility of ionized hydrogen, than the corresponding basic substances. Thus acid phosphates are separated from alkaline blood."

"In harmony with this idea is the observation of Tessier (27), 1877, of the inverse relations in the excretion of glucose and phosphates, the significance of which probably lies in accentuation of the state of acidosis during diminished glucose outgo."

Folin (28),1905, states that the phosphates of clear acid urine are all monobasic, the acidity of such urines being ordinarily greater than the acidity of all the phosphates, the excess being due to free organic acids. He believes that the precipitate formed with barium chloride, which is ordinarily considered to include the diacid phosphates, in reality contains phosphorus only as an impurity in the abundant precipitate of barium sulphate.

Hammarsten (29), claims that phosphorus is found in urinary sediment as tri-calcic and tri-magnesium phosphates, in alkaline urines; as dicalcium phosphate, in neutral or faintly acid urines; and as ammonium magnesium phosphate,in urines which have become ammoniacal through fermentation.

Organic Phosphorus

"In a fasting experiment on himself Keller (30) found in a four day test that the amount of organic phosphorus in the urine increased daily, thus, 0.017; 0.029; 0.034 and 0.057 grams of P_2O_5. These increased amounts constituted increased percentage of the total urinary phosphorus, though this also increased from day to day. The significance of orgrnic phosphorus of the urine was not established. It did not vary regularly with the diet phosphorus, nor with the absorbed phosphorus, nor with the nitrogen metabolized. It was not influenced by Na_2HPO_4 ingested."

"Lepine (31), 1901, states that in fatty degeneration of the liver its high lecithin content is accompanied by an abnormal glycerophosphoric acid content of the urine, which may reach a value as high as twice the usual amount."

Mandel and Oertel (32),1902, observed no change in the organic phosphorus of the urine caused by foods rich in phosphorus.

"Henderson and Edwards (33), 1903, compared phosphorus figures on the urine by direct titration and after fusion. From the correspondence of the results they concluded that there was no organic phosphorus present."

Bornstein (34),1905, considered the origin of the organic phosphorus of the urine in metabolism experiments on himself. He concluded that overfeeding with protein did not increase the organic phosphorus of the urine, and although he did not determine the source of this organic phosphorus, he was of the opinion that it was of endogenous origin.

Symmers (35), 1904, states that exercise and diet do not effect the urinary excretion of phosphorus in the organic form. The organic phosphorus he says may vary from a very small quantity to about nine tenths of the total phosphorus. He finds the excretion of organic phosphorus to be somewhat rythmical, periods of excessive secretion alternating with what may be either retention or diminished production. It is pronounced in lymphatic leukaemia and in nervous diseases. Total phosphorus was determined by titration with uranium nitrate after fusion, and inorganic phosphate by uranium nitrate titration without fusion, organic phosphate was reckoned by the difference.

Plimmer, Dick, and Lieb (36), 1909-10, found inorganic phosphorus to constitute 90 - 100% of the whole, its amount depending on the intake of P_2O_5. Organic phosphorus excretion they found very irregular, and not dependant on the diet, and concluded that it must be of endogenous origin.

Mathison (37), 1910, determined organic phosphorus on five healthy persons on 3 - 6 consecutive days, and in the urine of one person as affected by exercise and by the ingestion of glycerophosphoric acid, and sodium glycerophosphate. Organic phosphorus was found normally present, usually in amounts greater than .1 grams per day, though occasionally it fell below, and once reached .3 grams of P_2O_5.

The organic phosphorus outgo was not influenced by the ingestion of glycerophosphoric acid.

Summarizing the evidence;"urine may contain organic phosphorus as glycerophosphoric acid and as phosphocarnic acid. This fraction is variable in amount from a very small to a large part of the whole, and is too large a factor to ignore in any quantitative work. This organic phosphorus increases after fast, chloroform anaesthesia, morphine injection, and in pathological conditions. Its significance is not known. Its amount seems slightly to increase after the ingestion of glycerophosphates, but there is no marked or constant effect of the constituents of the food, either phosphorus containing or otherwise, or of the amount of phosphorus absorbed, or of the nitrogen metabolism on this excretion. It appears to be to some extent an individual characteristic, though temporary physiological states seem to be the dominant factors in its quantitative variation."

The feces are the main outgo of metabolized and unmetabolized phosphorus in herbivora, while among the carnivora and omnivora the main outgo is through the urine.

"Sick (38),1857, found that the ingestion of sodium phosphate increased urinary phosphorus by more than the added amount, with a decrease of earth phosphates and an increase of alkali phosphates."

"Von Hoesslin (39), 1909, in experiments with dogs studied the effects of sodium chloride on metabolism. He concluded that addition of sodium chloride to the food increased the outgo of phosphorus, especially in the urine. The excretion of tertiary sodium phosphate by the kidneys is increased by water, and still more by sodium chloride intake. The phosphorus outgo, especially in the urine, is influenced by overheating. Phosphates added to the diet cause diuresis and an increase in the percentage content of the urine in phosphorus."

Summary: Ingestion of acids or acid salts, or the formation of acids in the body, cause an increase in the calcium and phosphorus of the urine, and an increase in the proportion of acid phosphates to total phosphates. A decrease in the urinary phosphorus is caused by the alkaline earths. Sodium phosphate has a diuretic effect, and is eliminated promptly from the kidneys.

Inorganic Phosphates

"Lehmann (40), 1859, found in balance experiments with a calf that alkaline earth phosphates could be absorbed and retained."

Von Gohren (41), 1861, found that lambs could use calcium and magnesium phosphates.

"Blondlot (42), 1861, states that the administration of doses of .125 grams of sodium hypophosphite produces no phosphorous acid tests in the urine, but that doses of .5 grams give the characteristic green flame."

"Gamgee, Priestly, and Larmuth (43), 1876-77, compared the sodium salts of pyro, ortho, and metephosphoric acid by subcutaneous, intravenous, and oral administration to frogs, rabbits, and dogs. The ortho salt was found to be inert, the pyro salt markedly poisonous to the heart when introduced into the circulation, and the meta salt also poisonous, but to a less degree."

"Paquelin and Joly (44), 1877, administered 2 grams of sodium pyrophosphate daily for five days to a woman and found during this period and the following five days that apparently all of the pyrophosphate as pyrophosphoric acid was eliminated in the urine unchanged. The sodium pyrophosphate had a slight diuretic effect."

Paquelin and Joly (44), 1878, administered to a woman during five days a total of five grams of sodium hypophosphite. They concluded that the hypophosphites are excreted in the urine unchanged and have a diuretic effect.

" Vermeulin (45), 1884, studied the physiological action of hypophosphites, but without important results. He believes that they pass through the body unchanged. He determined hypophosphites in the urine by first removing the phosphates by uranium acetate precipitation and then determining total phosphates in a $KClO_3$ and HCl digest of the filtrate."

" Boddaert (46), 1896, found calcium and sodium hypophosphates rapidly excreted, apparently as such, in the urine, whether administered per os or subcutaneously, with rabbits, dogs, and men."

" Massol and Gamel (47), 1901, added sodium phosphate to a solution of calcium hypophosphite. The solution was then made alkaline and a precipitate of tricalcium phosphate was formed, leaving sodium hypophosphite in solution. The same reaction, they state, takes place in the intestine, and the tricalcium phosphate is lost to the organism. The sodium hypophosphite is absorbed, and excreted unchanged by the kidneys, there being no oxidation of phosphorous acid. Thus calcium hypophosphite removes calcium from the system in proportion to the amount fed."

Panzer (48), 1902, declared that "calcium hypophosphite fed to a dog is quickly and almost completely absorbed, passes through the organism without being held back anywhere, and is completely eliminated within twenty-four hours."

"Martinet (49), 1902 says that hyperacidity of the urine accompanied by abnormally small amounts of phosphates is frequently an index of hypochlorhydria of the stomach, and that insuch cases administration of phosphoric acid may promote digestion and check fermentation by its eupeptic and antiseptic action."

In 1905 Martinet (49) writes that out of forty-eight cases of dyspepsia, thirty-two cures have been accomplished by the use of phosphoric acid. It benefits the liver, increases urea excretion, causes indican to disappear, and relieves glycose urea of hepatic origin. It stimulates the hepato-pancreatic function, regulates intestinal functions, and suppresses diarrhoea.

"Joulie (50), 1904, administered phosphoric acid in rhumatism of horses. The dose was ten cubic centimeters diluted to one liter. The alkalinity of the blood is diminished, and the elimination of calcium phosphate by the kidneys is increased by such introduction of phosphoric acid.

"Delaini (51), 1911, submits data showing that human beings and dogs eliminate in the urine practically the whole amount of hypophosphite of sodium administered either per os or subcutaneously, but that rabbits otherwise dispose of perhaps twenty percent of the intake."

"Patta (52), 1910, administered to a dog, by injection, varying doses of sodium hypophosphite. If administered in large amounts, about half of the quantity introduced can be recovered, unoxidized, in the urine; when smaller amounts are administered, the proportion recovered is lower, being as low as one-sixth at times.

Hence it is maintained that the phosphorus of hypophosphites can be retained by the organism. "

Lecithin and Other Compounds of Glycerophosphoric Acid

"The phosphorus of lecithin and glycerophosphates may be absorbed from the alimentary tract and utilized in the tissue.. Lecithin administered subcutaneously may be retained, but there is some evidence that glycerophosphates under this condition are quickly eliminated by the kidneys."

" Lecithin added in the form of egg yolk to the milk diet of an infant appears to favor nitrogen and phosphorus retention and gain in weight."

"Egg yolk as compared with plasmon (a milk albumin preparation) appeared from experiments with dogs and guinea pigs, to favor the growth of the animal, and the development of the bones."

"As with other phosphorus compounds lecithin and the glycerophosphates are, in their metabolism,largely independant of nitrogen and calcium, at least during the limited period covered by most balance experiments."

"We have in these balance data no evidence of the possession by lecithin or glycerophosphates of any unusual nutritive or stimulative values, when added to the normal food of healthy animals. That they do possess a higher degree of usefulness in states of impoverishment, however, seems probable. Naturally, the apparent results of the administration of these compounds depend on the nutritive status of the subject, and on other dietetic treatments used for comparrision. In some states of nutritive derangement they possess life-saving capacity; in some other more favoradle circumstances they may be of no unusual value."

Relative Nutritive Values of Organic and Inorganic Phosphorus.

"W: S: Hall (53), 1896, from feeding experiments with mice came to the conclusion that salts organically combined with casein have a value greater than salts not so combined."

"Steinitz (54), 1898, conducted balance experiments on dogs to compare organic and inorganic phosphorus compounds as nutrients. Nutrose, a sodium-calcium-casein compound, vitellin, and myosin prepared from horse flesh were added, in different periods, to a basal ration of bacon, rice starch, and mineral salts; the casein preparation and the vitellin both containing organic phosphorus, and the myosin being at least practically free from organic phosphorus. Phosphorus was stored abundantly on the organic phosphorus rations, but the retention was almost nothing on the myosin ration, though in all cases, including the myosin ration the nitrogen was stored in considerable quantities. The salt mixture used with the organic phosphorus compounds contained chlorides and citrates only, while the salts used with the myosin were calcium, magnesium, and potassium phosphates, and sodium chloride, and ferric citrate. The experimental periods were 5 - 9 days each, following 8 - day fast periods."

"Rohmann (55), 1898, found, in balance experiments on a dog, with a diet of lard, rice starch, salt, and either a phosphorus free protein and a phosphate, or a phosphorus containing protein, that both nitrogen and phosphorus retention were favored by the phosphorus containing protein much more than by the phosphorus free protein and phosphates. With nutrose and vitellin the nitrogen retention was 31.0 and 42.2 percent respectively, of the intake; while with myosin and edestin the retention was but 0.10 and 0.11 percent of the intake. Likewise with the nutrose and vitellin the phosphorus retention was 8.8 and 20.9 mg. per day, while with myosin and eddstin the retention was 0.1 mg. per day in both cases."

"Leipziger (56), 1899, studied metabolism in a dog on a ration which was very low in organic phosphorus. The ration was composed of edestin, fat, starch, salt, beef extract, and water. The dog was fasted for six and ten days respectively, in the fore periods of two tests, and then fed for six days in each of the two main periods."

"The phosphorus intake on the two experiments was 1.874 and 2.022 grams per day, of which 0.016 and 0.022 grams was organic. The dog retained 0.0078 and 0.095 grams of phosphorus per day in these tests, the nitrogen and calcium balances being positive."

"Leipziger considered that his data did not afford evidence as to the condition in which phosphorus was retained, but thought that the synthesis of phosphorus containing protein from phosphorus free protein was improbable; also that the phosphorus retention was less than it would have been on a ration containing more phosphorized protein. The organic phosphorus content of this ration was sufficient to render it impossible to solve the problem of phosphorized protein synthesis except by negative results, which were not obtained."

"Zadik (57), 1899, conducted nitrogen and phosphorus balance experiments with dogs for the comparison of phosphorized proteins with phosphorus free proteins and inorganic phosphates. The compounds of interest were casein, vitellin, and edestin.

The basal ration was composed of starch, bacon, sodium citrate or carbonate, and a salt mixture of phosphates, chlorides, magnesium citrate, and sugar. The numerical data contained numerous inconsistancies, on account of which they are not transcribed, but the errors do not seem to be of a degree to modify at all the significance of the results. With casein or vitellin there was a marked retention of phosphorus; with edistin and disodium phosphate there was always loss."

"Zadik concluded that the animal organism does not have the power to build from phosphorus free proteins and phosphates the organic phosphorus compounds necessary for the life of the cells. The organic phosphorus of casein and vitellin was, under his experimental conditions, at least, more useful than the inorganic phosphates; also the phosphorus of vitellin was stored in larger proportions to the intake than was the phosphorus of casein."

"Ehrlich (58), 1900, conducted five balance experiments with phosphorized proteins and with phosphorus free proteins plus inorganic phosphates. The results tend to show that the phosphorized proteins, casein and vitellin, have a greater usefulness to the animal, in the sense of favoring phosphorus retention, than does the phosphorus free edestin plus inorganic phosphates. The periods were 3 - 7 days in length. Certain unfavorable conditions render the results of doubtful value."

"Kornauth (59), 1900, compared synthetic "nucleins" prepared after the method of Liebermann, by the precipitation of egg albumin and blood serum albumin with metaphosphoric acid, with natural proteins in the form of meat, skin, aleuronate (a vegetable casein), casein, and conglutin (from lupines). The results on nitrogen equlibrium hardly warrant conclusions, but the evidence is quite satisfactory in showing that for maintainance of phosphorus equlibrium, very much more phosphorus in the so-called synthetic nucleins is necessary than of phosphorus in the natural proteins."

"Tunnicliffe (60), 1906, conducted balance experiments with two healthy children, aged respectively, 2 years, and 2 years and 10 months, comparing organic and inorganic phosphorus compounds, and their effects on nitrogen metabolism. From the data Tunnicliffe concluded (1) that in healthy children the addition of an organic phosphorus compound to the diet is followed by an increase in the amount of phosphorus assimilated by, and retained in the body; (2) that the addition of an organic phosphorus compound to the diet of children increases the amount of nitrogen assimilated; (3) that the addition of $Ca_3(PO_4)_2$ to the food did not increase the amount of phosphorus assimilated or retained by the child, nor did this compound exert any favorable influence upon the assimilation of nitrogen in the food; (4) that the phosphorus contained in the sodium glycerophosphate of casein (sanatogen) is practically entirely assimilated by the body."

"Forbes and Keith (18) suggest that the periods were too short to give results of great value, and that since the intake of nitrogen and phosphorus was greater in the organic phosphorus period than in the inorganic phosphorus period, these data do not furnish a fair basis for a comparison of the nutritive values of these compounds. At the same time it seems probable that the phosphorus of sanatogen is more useful than the same amount of phosphorus in $Ca_3(PO_4)_2$."

"Egbert Koch (61),1906, investigated the question of synthesis of phosphorus compounds from edestin and inorganic salts by the human being, by means of a feeding experiment on a man. The subject was in a normal state of nutrition throughout the experiment."

"In the first ration the phosphorus was present to a considerable extent as phosphoprotein, that is, as casein. In the second ration the phosphorus was present principally as inorganic phosphates. The intake of notrogen, phosphorus, and calcium was maintained practically constant, though the nitrogen intake in the second period was a little less than in the first."

In the inorganic phosphorus period the nitrogen storage was less, as was also the phosphorus storage, while the calcium balance changed from +0.354 grams to −0.183 grams. From these data the author concluded that his experiments supported the view that the human body cannot synthesize organic phosphorus from phosphorus free proteins and inorganic phosphates.

"Heubner (62),1911, conducted feeding experiments on young dogs, comparing phosphates and lecithin as sources of phosphorus for the organism. The dogs had been kept on a low phosphorus diet until their need for phosphorus was acute. Lecithin appeared much superior to phosphates as a source of phosphorus for growing dogs."

"Fingerling (63), 1912 showed that ducks on a ration which is low in organic phosphorus, produce eggs of normal content of lecithin and nuclein phosphorus. He therefore concludes that the animal organism possesses the ability to cover its requirement of phosphoric acid for the formation of lecithin and nuclein substances just as easily and completely with inorganic phosphates as with organic phosphates."

Forbes and Keith (18) believe that such a positive conclusion can be justified only with a ration free of organic phosphorus, and by demonstrating that the original content of the body and its parts was maintained without loss of organic phosphorus.

Smith and Mair (64), 1912, in studying the development of lipoids in the brain of the dog, concluded from the low content of the mother's milk in phosphatid, cerebrosid, and cholesterin, in comparison with the daily deposition of the same in the brain, that these compounds are synthesized from other substances in the milk.

Masslow (65), 1913, studied the biological significance of phosphorus for the growing organism by means of feeding, metabolism, tissue analysis, and enzyme estimation on young dogs. Normal feeding was compared with feeding on a phosphorus poor diet, and with feeding on a phosphorus poor food plus phosphates, glycerophosphates and lecithin, as well as casein and albumin.

Emaciation and death followed the phosphorus poor diet, with a diminution of the phosphorus content of the organs. The loss was mainly in organic phosphorus. The lipoids were the only organic phosphorus compounds which decreased. Apparently the brain and heart did not lose phosphorus, while the liver, intestines, muscles, bone marrow, and kidneys suffered the greates loss.

The ferment functions of the organs were markedly disturbed, the action of lipase, amylase, and diastase were depressed greatly, with a like tendancy as to catalase and nuclease. As with the phosphorus, the heart and brain suffer little while the liver suffers most.

Neither inorganic phosphates nor glycerophosphates prevented the impoverishment on a low phosphorus diet. Lecithin, however, caused an enriching of the organism in phosphorus,,especially in organic compounds other than lecithin. The greatest improvement was in the visceral organs. The ferments were also stimulated to greater action.

Exclusive milk diet, maintained beyond the period normal for such food brought about conditions similar to the low phosphorus diet.

Fingerling (66), 1913, in experiments on goats fed phytin, lecithin, nuclein, nucleic acid, and disodium phosphate and found no essential difference in the utilization of the phosphorus in the different forms.

Durlach (67),1913, ran feeding and balance experiments with young dogs. They were held for a time on a low phosphorus diet. Comparisons were then made of monosodium phosphates,and monopotassium phosphates, with Merk's ovo-lecithin and with a mixture of monopotassium phosphate, lecithin, sodium phytate, casein and sodium nucleate. The results were not conclusive, but seemed to point to the superior nutritive power of the lecithin inasmuch as two of the three dogs which received lecithin were the only ones to survive the experiment.

If organic phosphorus compounds are superior to the inorganic phosphorus compounds it may possibly be due to the greater complexity of organization. That is, in the case of those compounds which are absorbed in part or with only partial cleavage, the organism is spared a considerable amount of synthetic activity. The maintainance of the nitrogen equlibrium seems to be appreciably more efficient with the less completely split proteins than with the products of complete cleavage. This principal may be operative also with some organic phosphorus compounds.

Forbes (17), 1915, of the Ohio State Experiment Station, ran several experiments using those organic and inorganic phosphorus compounds which are most commonly used in supplementing the diet or as medicines. A study of the literature during the investigation revealed more evidence in favor of the superior nutritive value of lecithine and phosphoproteins than of any other of the compounds included in the investigation. In a series of experiments run on pigs, hypophosphites, hypophosphites and nucleic acid, glycerophosphates, phytin, and phosphates were used. In all ways, general activity, bone strength, appetite, etc. the pigs receiving the glycerophosphates were very superior, while those receiving the hypophosphites were the inferior. As a result of a fifth experiment, it is stated that while these pigs were fed as much phosphorus in the form of phosphates as they would tolerate, and while they were unable to take calcium carbonate when added to the diet, the pigs lost in the percent of calcium, magnesium, and phosphorus in the skeleton during the experiment: that is with a low phosphorus ration it seems to be impossible to make up the dificiency of phosphorus by the addition of readily soluble phosphates in the pure form.

However a final study seemed to point out that when added in the usually chemically pure forms to such low phosphorus basal rations as were used, composed of comparatively simple manufactured products of plants and animals, these five compounds do not differ in their nutritive effects upon the gross composition of the growth of swine except in so far as affected by the relative tolerance of swine toward these preparations, and the consequent influence on the spirits and activity of the animal.

The limit of tolerance of the pigs for glycerophosphates was not reached in any of these tests. Their limit of tolerance for all of the other compounds was reached unmistakably.

In order of decreasing acceptability to swine Forbes (17) would rate these compounds, when fed in amounts furnishing equal amounts of phosphorus, in the following order: glycerophosphates, phosphates, phytin, nucleic acid, (from yeast) and hypophosphites.

From the great difficulty experienced in the feeding of yeast nucleic acid and phytin, as well as the related compounds from wheat bran, it is concluded that the isolation of such compounds from natural products alters, at least, their therapeutic effects in such a manner that it becomes impossible to state, from investigations of this sort on pure compounds, what may be their effect or nutritive value in their natural relationships in common food.

From these experiments Forbes (17) noted another advantage in having the phosphorus compounds in the organic form.

The ready solubility of the uncombined phosphates results in such a concentration in the digestive tract that nausea or catharsis results. Therefore, much larger amounts of phosphorus can be utilized if they are liberated in the normal way,by digestive cleavage, from organic complexes.

Summary

It is to be recognized at this point, that the evidence which is at hand is not as satisfactory as it could be wished, since with the gradual accumulation of knowledge, has come also the realization of the large number of difficulties, and the requirements of a positive solution.

It must also be remembered that in many experiments there has been no guarentee of the purity of the compounds used or any assurance that the influence of other useful compounds associated with the organic compounds used has been guarded against.

However one point seems to have been definitely established; namely, that only a small part of the total phosphorus must be organic, in order to permit growth and reproduction to go on, provided inorganic phosphorus be present in sufficient amounts. That organic phosphorus is absolutely essential to any animal has not been demonstrated nor is the proof,that inorganic phosphorus can serve all the purposes for which an animal needs phosphorus, complete. There is much evidence to imply that with some species at least, some organic phosphorus compounds are more useful than the inorganic phosphorus as far as being more readily and economically used is concerned, and of maintaining a higher state of vitality, the difference probably depending, in part at least on the fact of the partial absorption and utilization of organic phosphorus compounds as such without complete digestive cleavage.

The work on vitamines suggests that the demonstrations of the superior nutritive value of organic to inorganic phosphorus compounds may have been influenced by the presence of minute quantities of other beneficial materials occuring in association with them in natural substances and appearing as impurities in the isolated organic compounds. Whether this theory is actually true or not is of course unknown, but however the question may be ultimately settled, the studies made by these men would suggest that if the organic compounds are not of superior usefulness, or absolutely essential to the maintainance of growth, then other nutrients associated with them are essential, and therefore put a new emphasis on the value of organic foodstuffs over inorganic or artificially prepared nutrients.(18)

EXPERIMENTAL PART

General Description

The following work consists of a comparison of the urinary elimination of organic, inorganic, and total phosphates, following the ingestion of organic phosphorus, hypophosphites, and di-sodium phosphate, superimposed, during seperate periods, upon a low phosphorus diet. The subject was a healthy normal woman.

The experimental periods were eight, seven, and eight days in length respectively. In each case the added phosphorus was ingested with the morning meal on the fifth day.

The basal diet during the first two experiments consisted of soda crackers, egg white, butter, dates, and sugar, with a total phosphorus content of .1886 grams per day. This diet contained 45.58 grams of protein, and yielded 1826.58 calories per day.

Basal Diet

Food	Grams	Calories	Protein gms.	Phosphorus gms.
Egg White	200	121	29.18	.0026
Soda Crackers	113.5	472.5	11.2	.118
Butter	52	400	.52	.008
Cream	222.2	454.08	2.88	.010
Sugar	20	79	——	——
Dates	87	300	1.8	.048
Total	684.7	1826.58	45.58	.1866
Requirement		1500-2000	50-60	.88

Due to the difficulty of keeping the cream sweet, it was eliminated during the third period and more butter and sugar were substituted in its place. This gave a total phosphorus content of .1846 grams per day. The calorie and protein content were also lowered slightly, the former to 1819.9 and the latter to 43.22 grams per day. In adding the phosphorus in its different forms sufficient quantities were ingested to bring the dietary total for the fifth day up to approximately .88 grams, which is the minimum requirement for health.

Total phosphates in the urine were determined by the uranium acetate method. To 25 c.c. of urine, 5 c.c. of a buffer mixture of sodium acetate and acetic acid were added. This was heated to boiling and then titrated with uranium acetate to precipitate the phosphorus as uranium phosphate. During the first experiment potassium ferro-cyanide was used as an outside indicator. The color change with this indicator is a difficult one to distinguish. Therefore, in the second and third experiments, cochineal was used. It was added directly to the urine after precipitation had ceased. A change from red to green indicated the end point. The uranium acetate solution was standardized so that 1 c.c. was equivalent to about .005 grams of P_2O_5.

Inorganic phosphate was determined colorimetrically. 1 c.c. of urine was diluted to 100 c.c. Then 1,2,3, or 4 c.c. of this dilution, depending on the approximate amount of inorganic phosphate present, were placed in a test tube graduated at 10 c.c. At the same time 2 c.c. (0.02 mg.) of a standard monopotassium phosphate solution were placed in a similar test tube. To these were added 2 c.c. of a molybdate-sulphuric acid mixture, 1 c.c. of dilute stannous chloride solution, and water to the mark, and then mixed. The phosphomolybdate formed is reduced by the stannous chloride to form a blue colored compound which may be determined colorimetrically.

The organic phosphorus was computed as the difference between total phosphates and inorganic phosphorus, as P_2O_5.

Since the majority of investigators consider the difference between total phosphorus and total phosphates to be very small, as most of the phosphorus is eliminated in the phosphate form, the total phosphorus was not determined. +

The results of the three periods are tabulated in tables one, two and three. In table one are the results of the first period, in which the additional phosphorus was ingested as organic phosphorus in the form of egg yolk and casein. In table two the data are given for the second period, in which the additional phosphorus was fed as inorganic phosphorus in the form of compound syrup of hypophosphites. (This contains the hypophosphites of calcium, potassium, sodium, iron, and manganese, hypophosphorous acid, quinine, strychnine, sodium citrate, and sugar.) Table three contains the data for the third period, in which the additional phosphorus was ingested as inorganic phosphorus in the form of di-sodium phosphate.

+ The complete directions for making up the various solutions were obtained from Hawk and Bergeim, "Practical Physiological Chemistry", 875 - 876, P. Blakiston's Son & Co. Philadelphia.

The periods consisted of eight, seven, and eight days respectively. The first four days of each period constituted the preliminary period, phosphorus was added on the fifth day, and the remaining two or three days were allowed for the ingested phosphorus to be eliminated.

The daily elimination intervals were 8 A.M. - 12 M.; 12 M. - 4 P.M. ; 4 P.M.- 8 P.M.; and 8 P.M. - 8 A.M. Each sample of urine was analysed for total P_2O_5 and inorganic P_2O_5. Organic phosphorus was calculated from this by difference. The volume, specific gravity, and reaction were determined for each sample, and the color and turbidity noted.

Discussion of Results and Conclusion

From the comparison of results in table four, and from the graphs, the following conclusions may be drawn.

After the ingestion of organic phosphorus as egg yolk and casein, there is an increase in total phosphates, and inorganic phosphates approximately equal to the amount ingested, namely, .69 grams of phosphorus, or 1.58 grams of P_2O_5. Following the ingestion of di-sodium phosphate there is an increase in the excretion of organic phosphates equivalent to about two thirds of the amount actually ingested, but there is practically no increase in the inorganic phosphorus excreted. In the case of the hypophosphites there is apparently no increase in either the organic or inorganic phosphates. It is probably true that this form of inorganic phosphorus is excreted immediately, unchanged, as hypophosphites, but this was not determined.

Judging simply from this limited data it would appear that the ingestion of organic phosphorus increases both organic and inorganic phosphate elimination. This would indicate that such organic phosphorus is split during the process of digestion into two portions, one organic and the other inorganic. In the case of di-sodium phosphate there is practically no increase in the inorganic phosphate excretion, but all the increase is in the organic phosphate. This would indicate that the particular phosphate that was used, and possibly similar phosphates, are assimilated and metabolized so that the increased phosphorus excretion following such an ingestion is found to be almost wholly in organic phosphate.

These results seem to bear out the results of experiments performed by Paquelin and Joly (44), Vermeulin (45), Boddaert (46), Panzer (48), and Delaini (51) on hypophosphites, in which they found the hypophosphites to be rapidly excreted, unchanged, in the urine.

So far as the organic phosphorus and the inorganic phosphates are concerned the results vary, although the later experimenters feel that the inorganic phosphates can be utilized at least to satisfy some of the body requirements.

PHOSPHORUS EXCRETION

Table 1

Organic Phosphorus
Ingested as Egg Yolk and Casein = 1.58 gm. P_2O_5

Samp.	Total P_2O_5	Inorg. P_2O_5	Org. P_2O_5	Vol.	Sp.Gr.	Reaction	Color	Turb.
Day 1								
8-12	.1309	.0814	.0494	160	1.18	Neutral	Straw	Slight
12-4	.1605	.2398	———	460	1.03	Acid	Light Straw	Slight
4-8	.2062	.1722	.0341	315	1.07	Acid	Light Straw	Slight
8-8	.4740	.2341	.2399	460	1.15	Sl.Acid	Light Amber	Slight
Comp.	.9514	.7752	.1760	1395	1.09	Acid	Straw	Slight
Day 2								
8-12	.1154	.0216	.0938	155	1.20	Neutral	Light Amber	Slight
12-4	.1606	.0264	.1342	210	1.11	Acid	Straw	Slight
4-8	.1775	.0247	.1527	175	1.15	Acid	Straw	Slight
8-8	.3602	.0903	.2698	640	1.11	Neutral	Light Amber	Slight
Comp.	.7264	.2002	.5262	1180	1.16	Neutral	Straw	Slight
Day 3								
8-12	.0470	.0364	.0106	220	1.15	Neutral	Straw	Slight
12-4	.2102	.1594	.0508	(100	1.30	Acid	Amber	Slight
4-8				(60	1.23	Acid	Straw	Slight
8-8	.6240	.1739	.4501	820	1.09	Acid	Straw	Slight
Comp.	.5652	.2036	.3626	1200	1.10	Acid	Straw	Slight
Day 4								
8-12	.0135	.0315	———	195	1.16	Neutral	Straw	Slight
12-4	.1592	.0321	.1270	(95	1.30	Acid	Amber	Slight
4-8				(75	1.13	Acid	Amber	Heavy
8-8	.2182	.0403	.1780	255	1.20	Acid	Amber	Slight
Comp.	.2873	.0915	.1958	620	1.21	Acid	Straw	Slight

PHOSPHORUS EXCRETION

Table 1 Con't.

Samp.	Total P_2O_5	Inorg. P_2O_5	Org. P_2O_5	Vol.	Sp-Gr.	Reaction	Color	Turb.
Day 5, Organic Phosphorus Added								
8-12	.3308	.0152	.3156	(120	1.26	Acid	Amber	Slight
12-4				(165	1.21	Acid	Amber	Slight
4-8	.3449	.0172	.3277	285	1.14	Acid	Straw	Slight
8-8	.5573	.4863	.0710	890	1.12	Acid	Straw	Slight
Comp.	1.8250	.7603	1.0647	1460	——	Acid	Straw	Slight
Day 6								
8-12	.1544	.0911	.0632	175	1.20	Acid	Light Straw	Slight
12-4	.2600	.0202	.1395	(320	1.12	Acid	Straw	Slight
4-8				(60	1.28	Acid	Straw	Slight
8-8	.2491	.1313	.1178	525	1.17	Acid	Straw	Slight
Comp.	.4933	.6127	——	1070	1.14	Acid	Straw	Slight
Day 7								
8-12	.0389	.0421	——	145	1.21	Neutral	Straw	Slight
12-4	.0814	.0443	.0371	235	1.16	Acid	Straw	Slight
4-8	.0643	.0377	.0266	200	1.15	Acid	Straw	Slight
8-8	.1240	.0797	.0443	505	1.17	Acid	Straw	Slight
Comp.	.2978	.1858	.1120	1085	1.15	Acid	Straw	Slight
Day 8								
8-12	.0477	.0021	.0456	225	1.15	Alkaline	Straw	Heavy
12-4	.0481	.0365	.0116	(120	1.23	Acid	Straw	Slight
4-8				(90	1.30	Acid	Amber	Slight
8-8	.0779	.0957	——	550	1.15	Neutral	Straw	Slight
Comp.	.1736	.1631	.0106	985	1.16	Neutral	Straw	Slight

PHOSPHORUS EXCRETION

Table 2

Inorganic Phosphorus

Ingested as Hypophosphites ≡ 1.51 gm. P_2O_5

Samp.	Total P_2O_5	Inorg. P_2O_5	Org. P_2O_5	Vol.	Sp.Gr.	Reaction	Color	Turb.
Day 1								
8-12	.1862	.1518	.0344	235	1.21	Sl.Alk.	Straw	Slight
12-4	.2586	.2018	.0568	375	1.10	Acid	Straw	Slight
4-8				(105	1.28	Acid	Straw	Slight
8-8	.8998	.3408	.5590	(535	1.16	Sl.Acid	Straw	Slight
Comp.	1.3048	.6816	.6231	1250	1.18	Acid	Straw	Slight
Day 2								
8-12	.1192	.0208	.0984	165	1.22	Sl.Alk.	Straw	Slight
12-4				(100	1.24	Acid	Straw	Slight
4-8	.2761	.0504	.2257	(70	1.32	Acid	Straw	Medium
8-8	.7398	.0999	.6399	615	1.13	Acid	Straw	Slight
Comp.	.5711?	.1381	.4330	915	1.13	Acid	Straw	Slight
Day 3								
8-12				(105	1.28	Acid	Light Amber	Slight
12-4	.2772	.0365	.2407	(105	1.25	Acid	Straw	Slight
4-8				(110	1.18	Acid	Straw	Slight
8-8	.9791	.1350	.8441	(805	1.10	Acid	Straw	Slight
Comp.	1.3163	.1522	1.1641	1125	1.12	Acid	Straw	Slight
Day4								
8-12	.1370	.0435	.0935	250	1.18	Sl.Alk.	Straw	Slight
12-4	.2604	.0549	.2055	215	1.08	Acid	Straw	Slight
4-8				(125	1.15	Acid	Straw	Slight
8-8	.7081	.0872	.6209	(595	1.14	Acid	Straw	Slight
Comp.	1.0802	.0780	1.0023	1185	1.17	Acid	Straw	Slight

PHOSPHORUS EXCRETION

Table 2 Con't.

Samp.	Total P_2O_5	Inorg. P_2O_5	Org. P_2O_5	Vol.	Sp.Gr.	Reaction	Color	Turb.
Day 5, Hypophosphites Added								
8-12	.1192	.0330	.0862	175	1.20	Neutral	Straw	Slight
12-4	.3063	.0747	.2316	(150	1.16	Acid	Straw	Slight
				(				
4-8				(100	1.20	Acid	Straw	Slight
8-8	.6449	.0847	.5603	425	1.18	Acid	Light Amber	Slight
Comp.	1.0642	.1648	.8994	850	1.19	Acid	Straw	Slight
Day 6								
8-12	.1119	.0617	.0502	205	1.15	Sl.Alk.	Straw	Slight
12-4	.1978	.0825	.1153	425	1.07	Acid	Straw	Slight
4-8	.0974	.0231	.0743	135	1.19	Acid	Straw	Slight
8-8	.5122	.0697	.4426	380	1.18	Acid	Straw	Slight
Comp.	.9618	.1992	.7626	1145	1.13	Acid	Straw	Slight
Day 7								
8-12	.0898	.0241	.0656	160	1.18	Alkaline	Straw	Slight
12-4	.2149	.0452	.1696	(210	1.16	Acid	Straw	Slight
				(				
4-8				(50	1.28	Acid	Straw	Slight
8-8	.6941	.0907	,6035	670	1.13	Acid	Straw	Slight
Comp.	.9647	.1868	.7778	1090	1.15	Acid	Straw	Slight

PHOSPHORUS EXCRETION

Table 3

Inorganic Phosphorus
Ingested as Na_2HPO_4 = 1.58 gm P_2O_5

Samp.	Total P_2O_5	Inorg. P_2O_5	Org. P_2O_5	Vol.	Sp.Gr.	Reaction	Color	Turb.
Day 1								
8-12	.0552	.0723	———	120	1.17	Neutral	Straw	Slight
12-4	.1768	.2094	———	340	1.13	Acid	Straw	Slight
4-8	.2188	.1918	.0270	335	1.06	Acid	Light Straw	Slight
8-8	.5434	.3076	.2358	470	1.15	Acid	Light Amber	Slight
Comp.	1.0114	.7894	.2220	1275	1.17	Acid	Straw	Slight
Day 2								
8-12	.0522	.0392	.0130	220	1.14	Neutral	Straw	Slight
12-4				(60	1.23	Acid	Amber	Slight
4-8	.2656	.0965	.1691	(100	1.30	Acid	Amber	Slight
8-8	.7632	.2576	.5056	450	1.19	Acid	Amber	Slight
Comp.	1.0657	.4527	.6130	830	1.20	Acid	Straw	Slight
Day 3								
8-12	.1331	.1083	.0248	(115	1.17	Acid	Amber	Slight
12-4				(55	1.33	Acid	Amber	Slight
4-8	.1966	.0630	.1336	110	1.25	Acid	Amber	Slight
8-8	.6555	.4295	.2260	750	1.10	Acid	Straw	Slight
Comp.	.9664	.5242	.4422	1030	1.14	Acid	Straw	Slight
Day 4								
8-12	.0523	.0458	.0065	80	1.27	Neutral	Amber	Medium
12-4				(50	1.32	Acid	Straw	Heavy
4-8	.1333	.0495	.0838	(45	1.32	Acid	Amber	Slight
8-8	.6281	.2442	.3839	530	1.16	Acid	Amber	Slight
Comp.	.7988	.4037	.3951	705	1.19	Acid	Amber	Slight

PHOSPHORUS EXCRETION

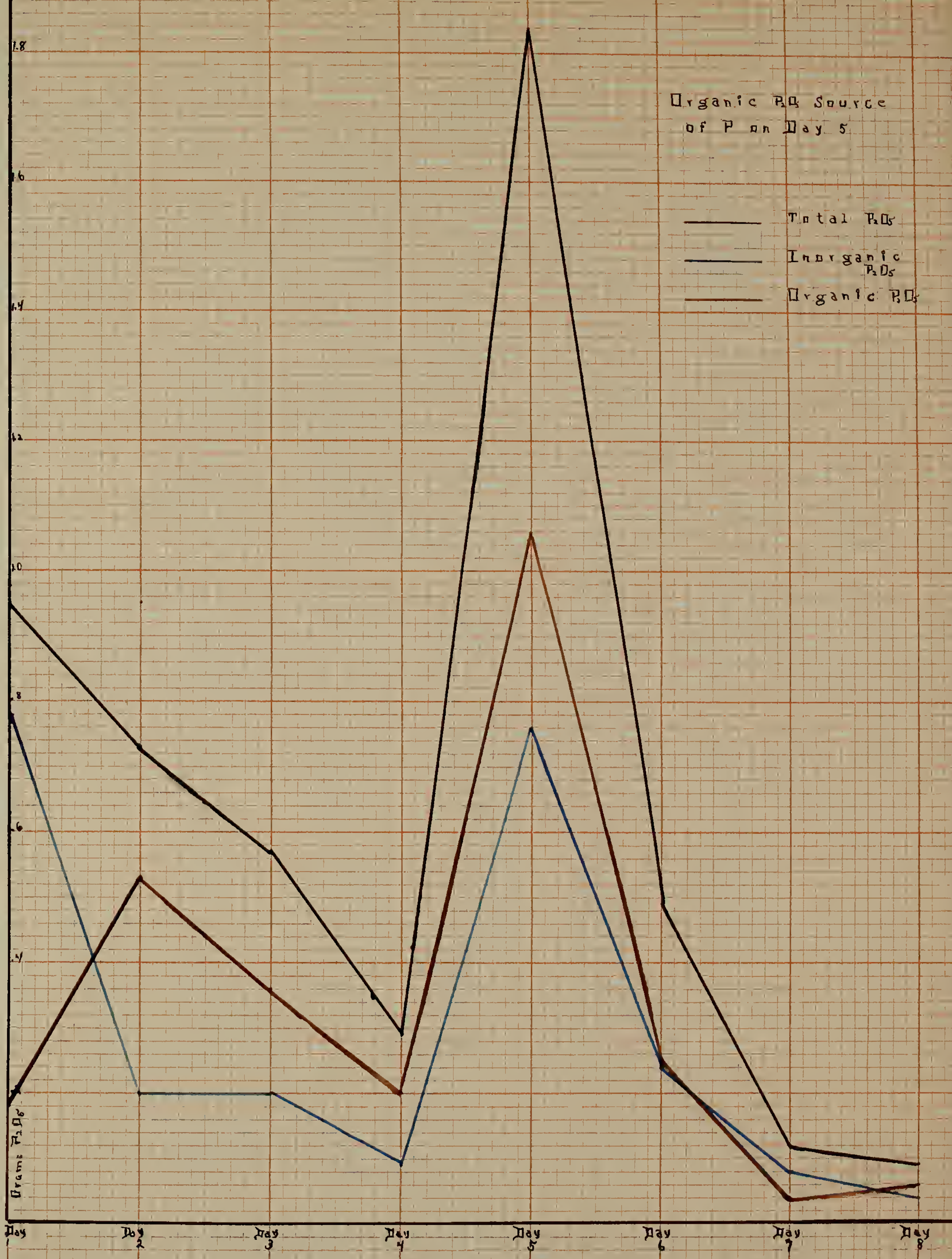
Table 3 Con't.

Samp.	Total P_2O_5	Inorg. P_2O_5	Org. P_2O_5	Vol.	Sp.Gr.	Reaction	Color	Turb.
Day 5, Na_2HPO_4 Added								
8-12	.0949	.0336	.0613	65	1.27	Alkaline	Amber	Medium
12-4	.3555	.0954	.2599	175	1.27	Neutral	Amber	Slight
4-8	.5307	.1582	.3715	290	1.16	Acid	Straw	Slight
8-8	.8586	.2080	.6426	345	1.20	Acid	Amber	Slight
Comp.	1.8486	.4661	1.3825	875	1.19	Acid	Amber	Slight
Day6								
8-12				(65	1.25	Acid	Amber	Slight
12-4	.1211	.1196	.0015	(190	1.10	Acid	Straw	Slight
4-8	.2152	.0781	.1371	150	1.24	Acid	Amber	Slight
8-8	.7379	.3518	.3861	645	1.13	Acid	Amber	Slight
Comp.	1.0763	.5583	.5180	1050	1.17	Acid	Amber	Slight
Day 7								
8-12	.0417	.0122	.0295	200	1.10	Neutral	Very Lt.Straw	Slight
12-4	.1096	.0209	.0887	350	1.11	Neutral	Straw	Slight
4-8	.0886	.0145	.0741	265	1.12	Acid	Straw	Slight
8-8	.5689	.1612	.4077	380	1.19	Acid	Amber	Slight
Comp.	.8198	.1841	.6357	1195	1.15	Acid	Straw	Slight
Day 8								
8-12	.0903	.0128	.0775	215	1.22	Neutral	Straw	Medium
12-4	.0201	.0148	.0053	155	1.09	Neutral	Straw	Slight
4-8	.2035	.0543	.1492	275	1.19	Acid	Straw	Slight
8-8	Lost							
Comp.	.2903	.0694	.2289	645	1.17	Neutral	Straw	Slight

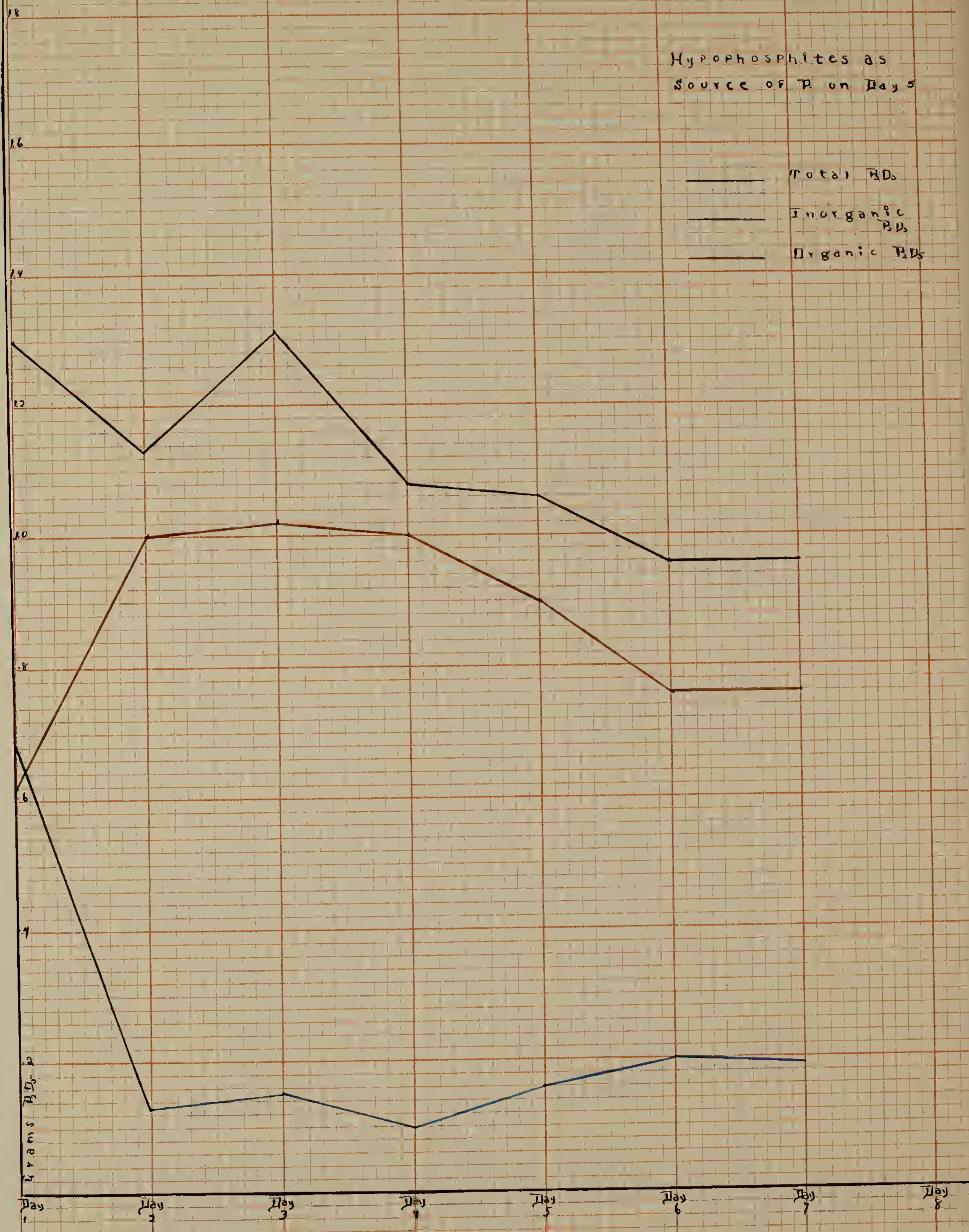
COMPARISON OF RESULTS

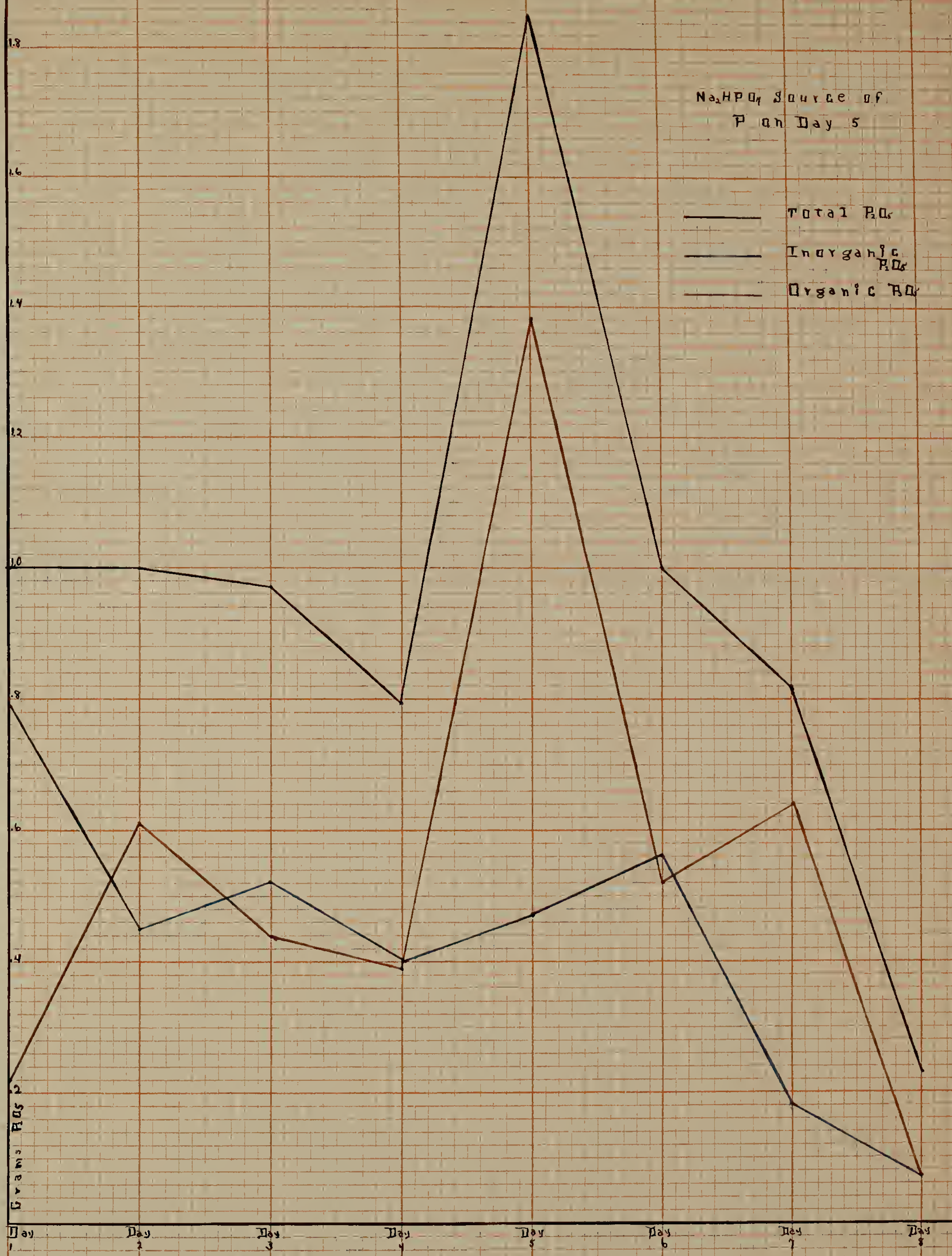
Table 4

Day	ORGANIC Total P_2O_5	ORGANIC Inor. P_2O_5	ORGANIC Org. P_2O_5	HYPOPHOSPHITE Total P_2O_5	HYPOPHOSPHITE Inor. P_2O_5	HYPOPHOSPHITE Org. P_2O_5	Na_2HPO_4 Total P_2O_5	Na_2HPO_4 Inor. P_2O_5	Na_2HPO_4 Org. P_2O_5
1.	.9514	.7752	.1762	1.3045	.6816	.6231	1.0114	.7895	.2220
2.	.7264	.2002	.5262	1.1351	.1381	.9970	1.0657	.4527	.6180
3.	.5652	.2036	.3626	1.3163	.1522	1.1641	.9664	.5242	.4422
4.	.2873	.0915	.1958	1.0802	.0780	1.0023	.7988	.4037	.3951
5.	1.8250	.7603 .6127?	1.0647	1.0642	.1648	.8994	1.8486	.4661	1.3825
6.	.4933	.2426	.2507	.9618	.1992	.7626	1.0763	.5883	.5180
7.	.1240	.0797	.0443	.9647	.1868	.7778	.8198	.1841	.6357
8.	.1736	.1631	.0106						
8. -(8-8)	.0958	.0386	.0572				.2903	.0684	.2269



Organic P2O5 Source of P on Day 5
Total P2O5
Inorganic P2O5
Organic P2O5
Grams P2O5
1.8
1.6
1.4
1.2
1.0
.8
.6
.4
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Day 8


Hypophosphites as
Source of P. on Day 5
Total P_2O_5
Inorganic P_2O_5
Organic P_2O_5
1.6
1.4
1.2
1.0
.8
.6
.4
.2
Grams P_2O_5
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Day 8


Na_2HPO_4 Source of P on Day 5
Total P_2O_5
Inorganic P_2O_5
Organic P_2O_5
1.8
1.6
1.4
1.2
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Grams P_2O_5
Day 1
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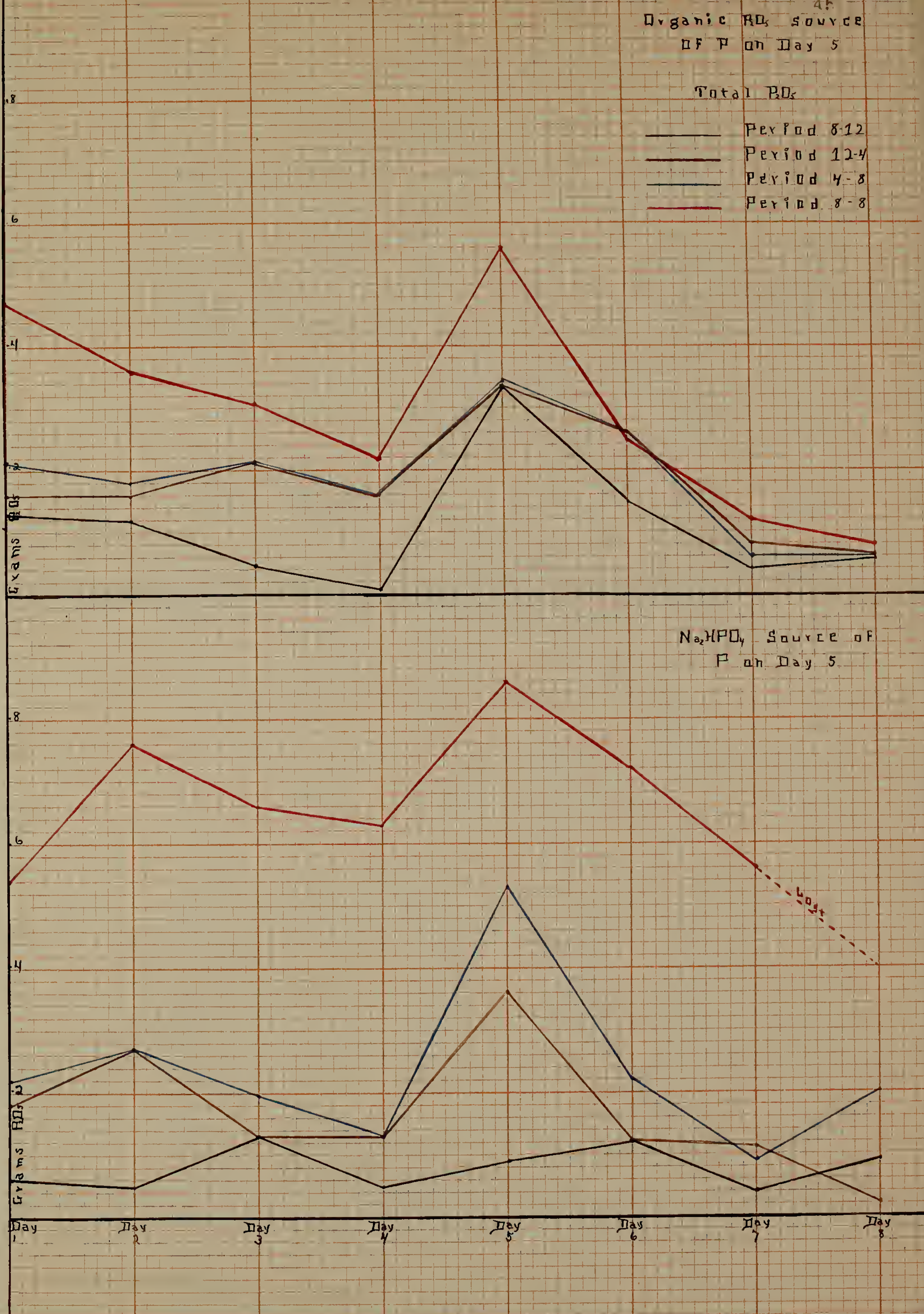
Organic P2O5 Source
of P on Day 5
Total P2O5
Period 8-12
Period 12-4
Period 4-8
Period 8-8
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Grams P2O5
Na2HPO4 Source of
P on Day 5
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Grams P2O5
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Day 8

Organic P_2O_5 Source of P on Day 5
Inorganic P_2O_5
Period 8-12
Period 12-4
Period 4-8
Period 8-8
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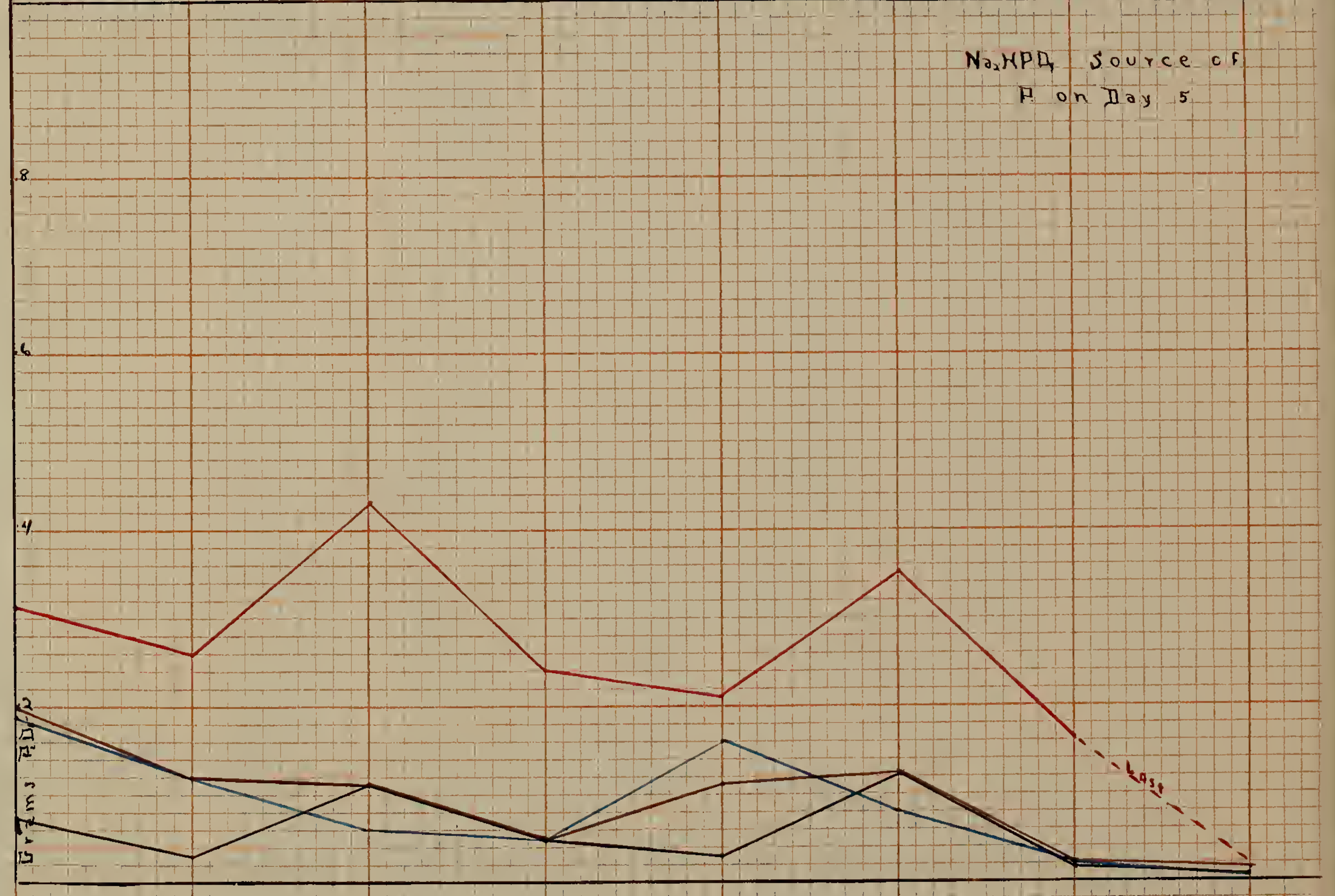
Grams P_2O_5



Na_2HPO_4 Source of P on Day 5
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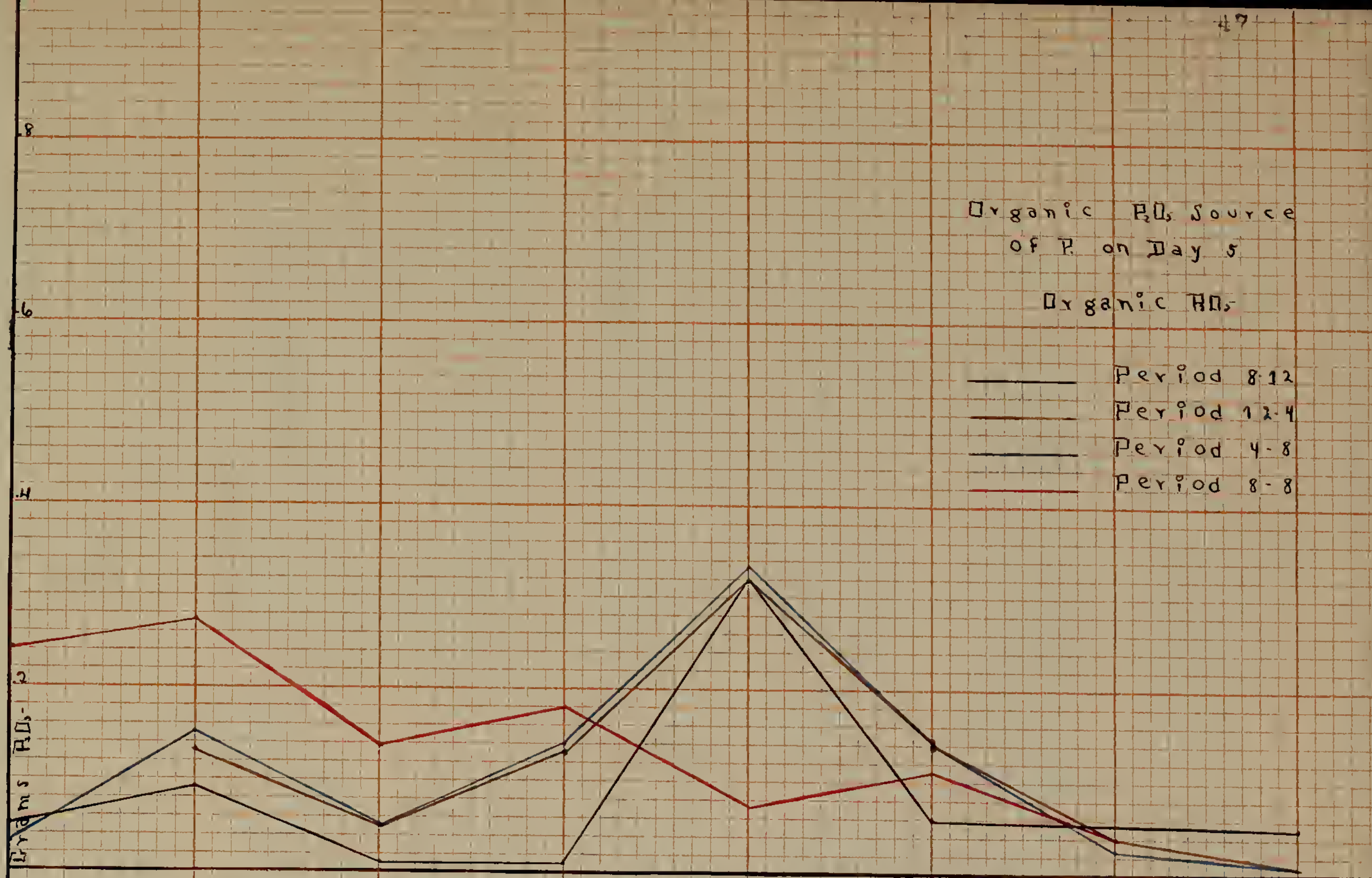
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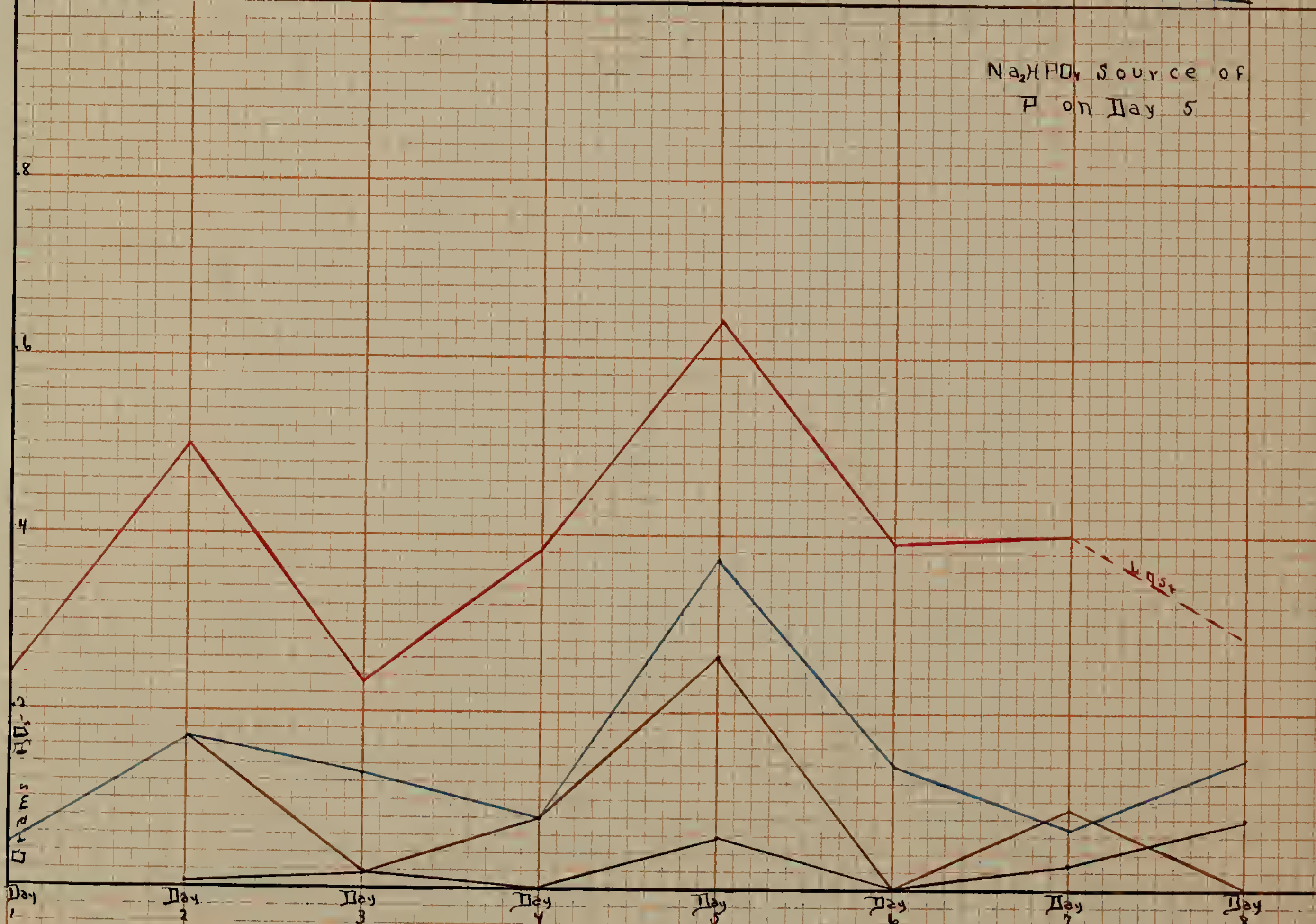
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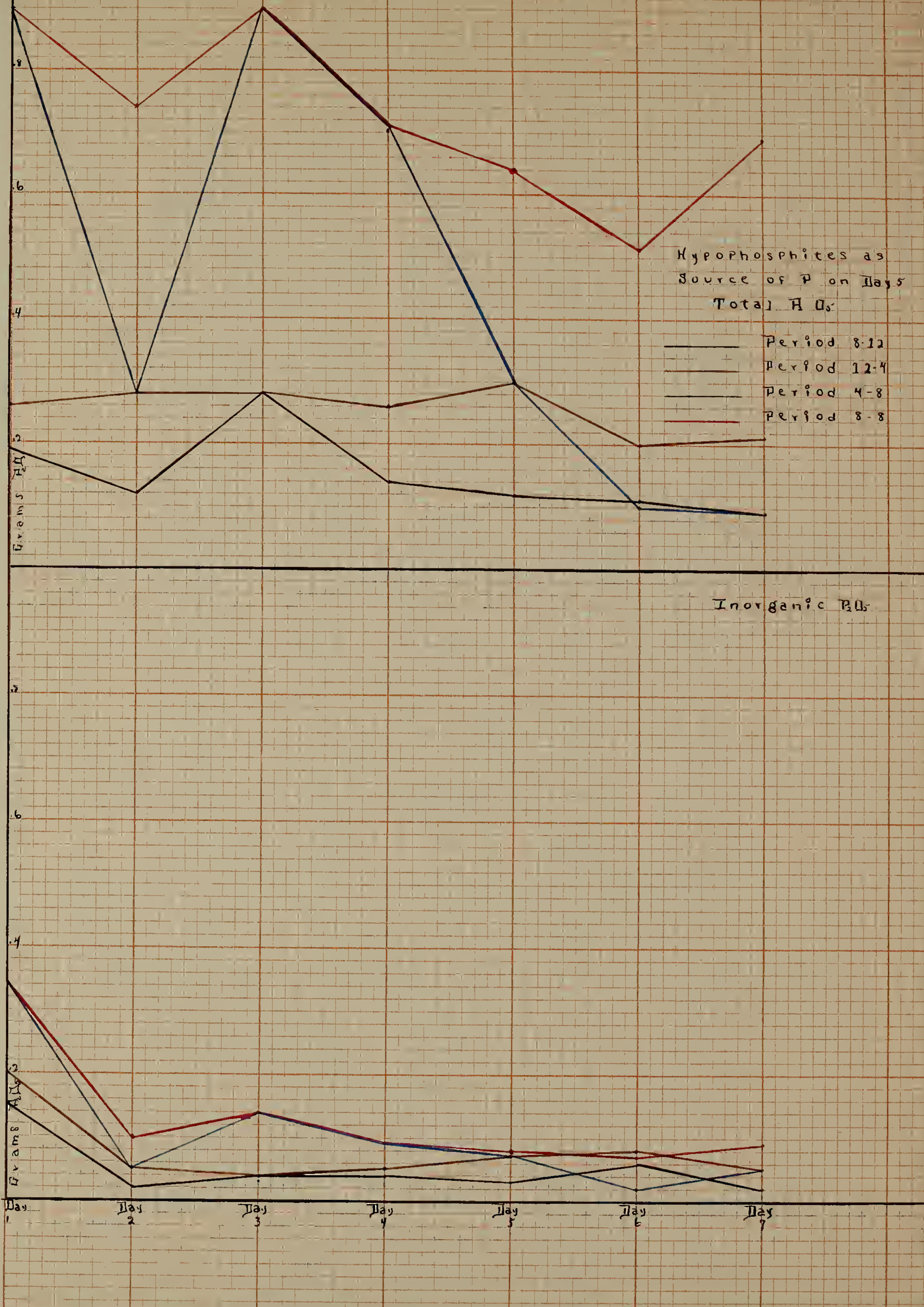
Hypophosphites as
Source of P on Days
Total P2O5
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Period 4-8
Period 8-8
Inorganic P2O5
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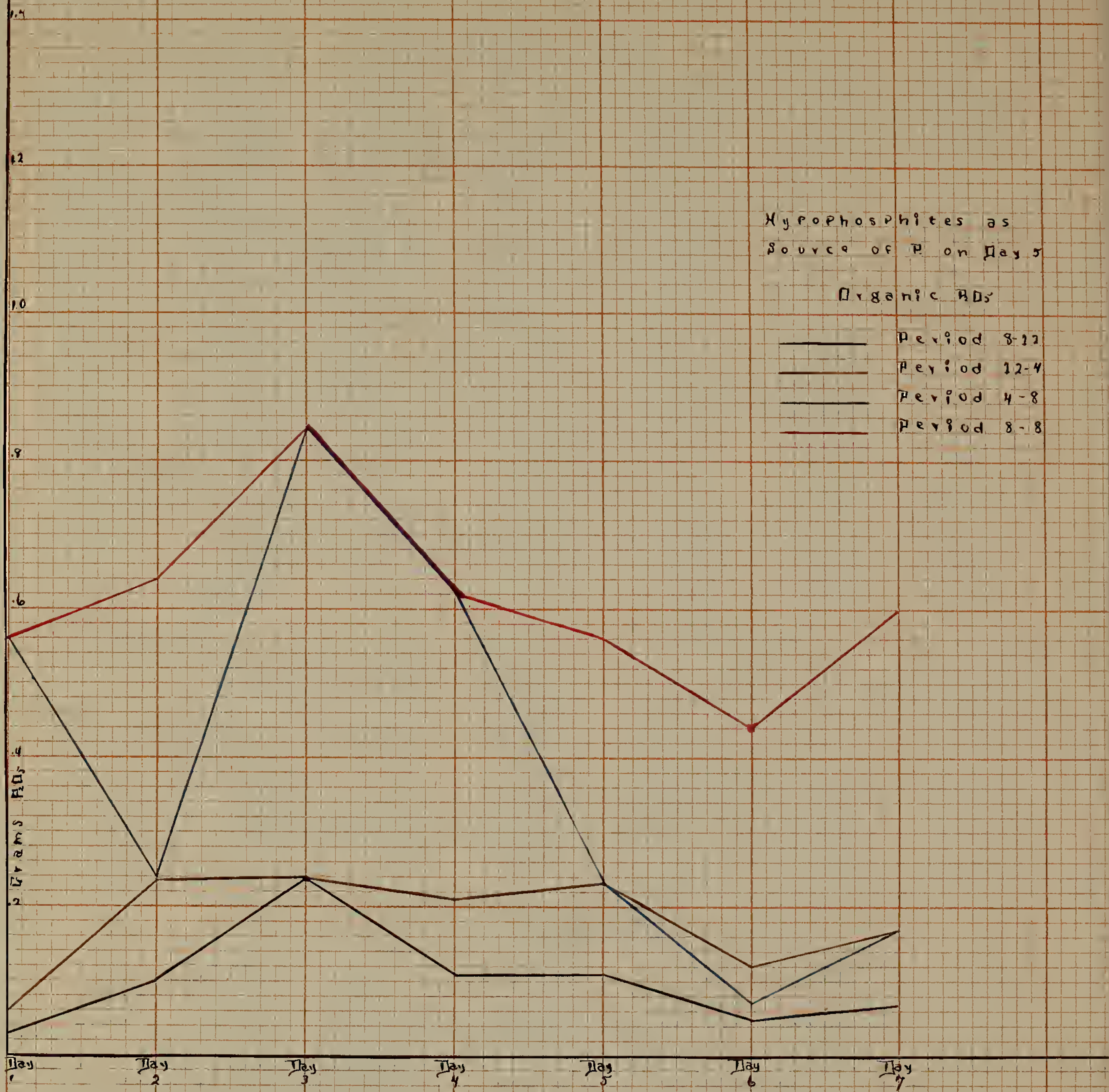
Hypophosphites as
Source of P on Day 5
Organic P_2O_5
Period 8-12
Period 12-4
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Period 8-8
Grams P_2O_5
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